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Yeasts from the North Sea and Amoco Cadiz Oil

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Abstract

The species and densities of yeasts isolated from North Sea waters before and after the production of oil were compared. *Debaryomyces hansenii* was the predominant species in both sets of samples, but after oil production, *Candida guilliermondii*, a hydrocarbonoclastic yeast, was more commonly isolated and the frequency of *Aureobasidium pullulans* decreased. Relatively few fungi were isolated from Amoco Cadiz oil collected twelve days after the start of the spill. The highest densities were obtained from the oil samples when they were first emulsified in a Tween 80-seawater solution. It is suggested that the high concentrations of aromatics in the unweathered Iranian crude, which constituted about half the spilled oil, were inhibitory to the yeast flora.

Introduction

Yeasts have been shown to be of widespread occurrence in the North Sea with population densities ranging from < 10 to >3,000 cells/ liter (Meyers *et al.* 1968). Increased densities of the predominant species, particularly *Debaryomyces hansenii* , appear to be associated with nutrient maxima related to thermoclines, phytoplankton blooms or surface films (Meyers *et al.* 1968, Crow *et al.* 1975). Hydrocarbonoclastic activities are widespread among yeasts and increased populations of yeasts have been reported in the vicinity of spilled oil (Scheda and Bos 1966, Ahearn *et al.* 1971, Meyers and Ahearn 1974, Crow *et al.* 1974, Ahearn *et al.* 1976). The development of offshore oil production in the North Sea has prompted us to reexamine the yeast flora. During 1976, one of us (S.A.C.) participated in a cruise of the R.V. Friedrich Heincke (Biologische Anstalt, Helgoland) in the North Sea to study the distribution and species of yeasts.

On March 16, 1978, the disabled supertanker Amoco Cadiz laden with about 223,000 tons of light Arabian and Iranian crudes grounded on the Brittany coast near Portsal, France. The entire oil cargo was lost from the vessel in rough seas within two weeks with about 30 % washed to the adjacent 300 to 350 km of shore. The general area polluted with oil is characterized by alternating sandy and rocky shores exposed to high tidal ranges (5–9 m) and currents, but several submerged estuaries (abers) and several marshes (particularly Lannion Marsh) also were severely oiled. Between 28–30 March 1978, samples of crude oil and mousse (water in oil emulsion) from tidal pools and beaches near Portsal, France were collected. These samples were examined for their associated microflora with particular emphasis given to the yeasts. The species patterns obtained were compared with those from the earlier studies of yeasts from the North Sea.

Materials and Methods

Water samples from the North Sea were collected from 35 stations in open waters located between 54°N, 8°E and 66°40'N, 10°E. The collection of samples and the methods of isolation and identification of yeasts were as described in an earlier study (Crow *et al.* 1975).

Samples from the Amoco Cadiz spill were of three types; a viscous brown-black crude with apparently low water of emulsion, a brown mousse, and surface film collected from tidal pool water. Three samples of each were collected in sterile 10 ml vials from various sites within 10 km north of Portsal harbor. Each sample was held at room temperature and periodically, over a 6 month period, 0.1 ml was cultured in 20 ml of enrichment broths and 0.1 ml was diluted 1:10 in a 0.1 % Tween 80 sea water solution and inoculated onto Bushnell-Haas (Difco) agar with 0.01 % hexadecane, mycological agar (Difco) prepared with sea water, and marine agar (Difco). The enrichment broths were: filtered sea water with a 0.01 %

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yeast extract, sea water with 0.07 % $(NH_4)_2SO_4$ and sea water with both the $(NH_4)_2SO_4$ and yeast extract prepared with and without 0.1 % Tween 80. The enrichment broths were incubated at 20 and 26°C with agitation and every 3–4 days for up to 14 days 0.01 ml samples of the broth were inoculated onto the isolation agars by spread plate procedure. Representative colonies of microorganisms were isolated, identified and examined for their capacity to grow on various hydrocarbons according to procedures described earlier (Ahearn *et al.* 1971, Crow and Ahearn 1979).

Results and Discussion

The species of yeasts isolated from the North Sea are compared with those isolated in an earlier study prior to the development of oil production in Table I. The species patterns are quite similar with two notable differences. *Candida guilliermondii*, a yeast with moderate hydrocarbonoclastic activities, was the second most common isolate obtained in 1976, whereas only several atypical isolates initially identified as *Candida* sp. were obtained in 1964–66. In 1976, the incidence of *Aureobasidium pullulans* appeared reduced. Meyers *et al.* (1968) found yeasts at all of twelve stations in 99 % of 84 samples. The majority of samples contained between 35–50 cells/l with a maximum density of >3,000 cells/l. In 1976, yeasts were isolated from 100% of the surface samples collected at the 35 stations and from 28 of the 35 samples collected at 10 m. Yeast densities at the surface averaged 76 cells/l and 35 cells/l at 10 m.

Tab. I. Yeasts isolated from the North Sea

1964–66[a]	Incidence[b]	1976	Incidence
Debaromyces hansenii	38	*D. hansenii*	46
Rhodotorula rubra	29	*Candida guilliermondii*	29
Aureobasidium pullulans	23	*Cryptococcus laurentii*	14
Candida diddensii	18	*Rhodotorula rubra*	11
C. tropicalis	12	*Cr. albidus*	<10
R. pilimanae	11	*Cr. gastricus*	<10
Hanseniaspora uvarum	10	*Torulopsis candida*	<10
C. zeylanoides	<10	*R. graminis*	<10
C. obtusa	<10	*R. lactosa*	<10
C. krusei	<10	*C. tropicalis*	<10
C. lipolytica	<10	*C. parapsilos*	<10
C. silvicola	<10	*Kloeckera apiculata*	<10
C. tenuis	<10	*Rhodosporidium capitatum*	<10
Rhodosporidium infirmio-miniata	<10	*Sporobolomyces roseus*	<10
Rhodotorula graminis	<10	*S. gracilis*	<10
Sporobolomyces roseus	<10	*S. albo-rusescens*	<10
Hanseniaspora californica	<10	*Aureobasidium pullulans*	<10
C. guilliermondii (Candida sp.)	<10		
Total Samples	84		70

[a]See, Meyers *et al.* 1967
[b]Percent occurrence in total samples

Relatively few species of fungi were isolated from the Amoco Cadiz oil (Tab. II). Direct inoculation of oil onto agar plates gave only a few colonies indicating that fungal populations were <5 colony forming units per 100 ml representing no more than two species. When these oil samples were vigorously agitated in a Tween 80 solution, densities in some samples ranged to 57 cells/ml and others yielded up to five different species. The greatest variety of species was obtained from the surface films.

The isolation of the yeasts from the oil samples suggested successional development of species. *Rhodotorula rubra* was isolated sporadically in the highest numbers during the first 2–3 months of study, particularly from the surface film samples. This species has been noted in bloom occurrences in various marine waters, but representative Amoco Cadiz and North Sea isolates gave negligible growth on hexadecane and crude oil fractions. During the first three months only an occasional isolate of *C. tropicalis*, *C. guilliermondii* and *Debaryomyces hansenii* was obtained from the brown-black oil and the mousse samples. After three months *R. rubra* was no longer isolated from the samples and isolates of *C. tropicalis* and *C. guilliermondii* were obtained commonly. These latter species in contrast to *R. rubra* readily utilized hexadecane as a sole source of carbon for growth. None of the yeasts, including the single isolate of *C. lipolytica*, however, gave good oil emulsification or utilization comparable to that found for isolates of *C. lipolytica* and *C. maltosa* from other sources (Ahearn *et al.* 1976). Two morphologically distinguishable cultures of *Pseudomonas* which grew well in broth media with good oil emulsification were isolated sporadically from all except surface film samples.

All yeasts isolated from Amoco Cadiz oil represented species previously isolated from waters of the North Sea.

Tab. II. Fungi isolated from Amoco Cadiz oil

Species	Brown-Black Oil	Mousse	Surface film
Rhodotorula rubra	+	+	+
Debaryomyces hansenii	–	+	+
Candida tropicalis	+	+	+
C. lipolytica	–	+	–
C. guilliermondii	+	+	+
Aureobasidium pullulans	–	–	+
Penicillium sp.	+	+	+
Mucor sp.	–	–	+
Fusarium sp.	+	+	+

The yeasts, apparently in low numbers, showed a successional development in the oil to species with greater hydrocarbonoclastic activity.

In comparison to surface films and water samples examined in earlier work, the crude oil appeared selective and possibly inhibitory to normal marine yeast flora. Certain volatile hydrocarbons, dependent upon concentration, may be inhibitory for yeasts (Ahearn *et al.* 1971). The Iranian oil, which contained about 30 % naphthenes, has proven inhibitory to representative isolates of *Debaryomyces hansenii*, the most common yeast in North Sea waters in simple spot tests on nutrient agar. The presence of odorous volatile oil fractions at the shore adjacent to the wreck was noticeable even 10 days after the spill. The high concentrations of these volatile fractions may have markedly reduced the densities and species of yeasts brought into contact with the oil. The effects of various crude oils on the role of marine yeasts in the recycling of nutrients are poorly understood.

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References

Ahearn, D. G., S. A. Crow, N. H. Berner and S. P. Meyers. 1976. Microbiological cycling of oil in estuarine marshlands, p. 483–492. *In: Estuarine Processes I: Uses, Stresses, and Adaptation to the Estuary*. Academic Press, New York.

Ahearn, D. G., S. P. Meyers and P. G. Standard. 1971. The role of yeasts in the decomposition of oils in marine environments. *Dev. Ind. Microbiol. 12*: 126–134.

Crow, S. A., S. L. Bell, and D. G. Ahearn. The uptake of aromatic and branched chain hydrocarbons by yeast. *Bot. Mar. 23*: 117–120.

Crow, S. A., P. I. Bowman and D. G. Ahearn. 1975. Isolation of atypical *Candida albicans* from the North Sea. *Appl. Env. Microbiol. 33*: 738–739.

Crow, S. A., S. P. Meyers and D. G. Ahearn. 1974. Microbiological aspects of petroleum degradation in the aquatic environment. *La Mer 12*: 37–54.

Meyers, S. P. and D. G. Ahearn. 1974. Implication of yeasts and yeast-like fungi in marine processes. *Veröff. Inst. Meeresforsch. Bremerh. Suppl. 5*: 321–338.

Meyers, S. P., D. G. Ahearn, W. Gunkel and F. J. Roth, Jr. 1968. Yeasts from the North Sea. *Mar. Biol. 1*: 118–123.

Scheda, R. and P. Bos. 1966. Hydrocarbons as a substrate for yeasts. *Nature 211*: 660.